The EMBO Journal vol.6 no.12 pp.3795–3799, 1987

A human T lymphoblastic cell line lacks lamins A and C

M.N.Guilly, A.Bensussan[1], J.F.Bourge[1], M.Bornens and J.C.Courvalin

Centre de Génétique Moléculaire, CNRS, 91190 Gif-sur-Yvette, and [1]INSERM, U.93, Hôpital St Louis, F-75475 Paris Cedex 10, France

Communicated by S.Avrameas

Lamins A, B and C, the three major proteins of nuclear envelope, constitute a class of intermediate filament polypeptides. We have compared the amount of these polypeptides in two human cell lines, epithelial HeLa cells and T lymphoblasts KE 37. It was found that the three lamins were present in roughly equimolar stoichiometry in HeLa cells, while lamin B was the unique lamin component in T lymphoblasts. Moreover, 3-kb mRNA of lamin A and 2.1-kb mRNA of lamin C were detected wtih a human cDNA probe in HeLa cells but not in T lymphoblasts. These results suggest that (i) lamin B can build up the lamina structure in actively dividing somatic cells by itself, and (ii) lamin expression in lymphoid cells may be subject to important quantitative variations. Comparison of the lamin composition of human cloned T lymphocytes and Epstein–Barr virus-transformed human B lymphocytes confirmed this statement. The lamin B level was nearly equivalent in both cells but the content of lamins A and C varied to a large extent, being low in T cells and high in B cells.

Key words: differentiation/human lymphocytes/lamins

Introduction

The nuclear lamina is a proteinaceous layer apposed to the inner nuclear membrane. It is composed of a family of polypeptides, the lamins, highly conserved in evolution (for review see Krohne and Benavente, 1986). In mammals, three lamins (A, B and C) have been described with M_r ranging from 60 000 to 78 000. Lamins A and C (pI ~7) but not lamin B (pI ~5.4) have close sequence homology (Lam and Kasper, 1979; Gerace and Blobel, 1981). During mitosis the lamina meshwork is reversibly disassembled in parallel with phosphorylation of the lamins (Gerace and Blobel, 1980; Miake-Lye and Kirschner, 1985; Ottaviano and Gerace, 1985; Burke and Gerace, 1986). In view of their primary and secondary structure and self-association properties, lamins have recently been shown to constitute a class of intermediate filament (IF) polypeptides (Aebi *et al.*, 1986; Fisher *et al.*, 1986; Goldman *et al.*, 1986; McKeon *et al.*, 1986). As with other IFs, lamins might therefore be expected to display cell type-specific expression (Lazarides, 1982) and in fact this has already been observed in *Xenopus* (Krohne *et al.*, 1981; Benavente *et al.*, 1985; Stick and Hausen, 1985). Here we report the absence of lamins A and C in a human T lymphoblastic cell line and show that these two lamins are subject to important quantitative variations in other cells of the lymphoid lineage.

Results

Total proteins from human epithelial cells (HeLa) and human T lymphoblasts (KE 37) were analysed by immunoblotting using polyclonal antibodies specific for either lamin B (Guilly *et al.*, 1987) or lamins A and C (McKeon *et al.*, 1983). The results in Figure 1A show that HeLa cells were positive for the three lamins, while KE 37 cells apparently did not contain lamins A and C. The same result was obtained by immunoprecipitation

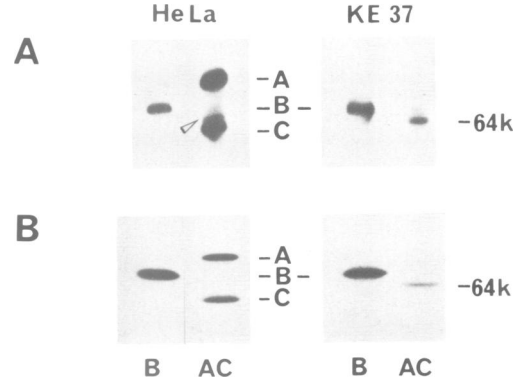

Fig. 1. Immunoblot (**A**) and immunoprecipitation (**B**) analysis of lamins. Two human cell lines were used: attached (HeLa) and unattached (T lymphoblasts KE 37). Nuclear proteins were probed with either serum F (lamin B-specific–left lanes) or serum LS -1 (lamins A + C-specific–right lanes). Note in KE 37 cells the absence of a signal for lamins A and C and the presence of a 64-kd protein. This protein is also detected in HeLa cells by immunoblotting (arrowhead). The stringent conditions used for immunoprecipitation of HeLa cells in this experiment (0.5% SDS) prevent the detection of the 64-kd minor component.

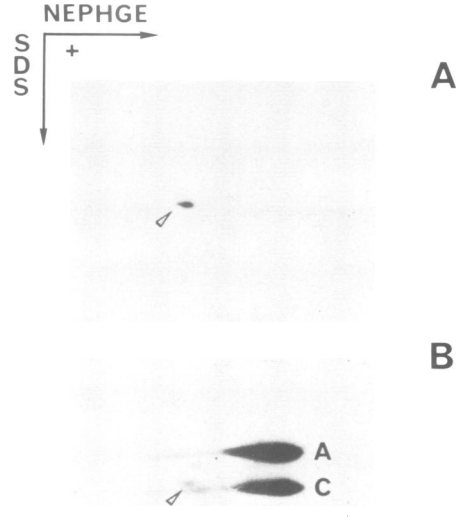

Fig. 2. Total proteins of KE 37 T lymphoblasts (**A**) and HeLa cells (**B**) were separated by non-equilibrium pH gradient electrophoresis, blotted, then probed with the anti-lamin A + C serum. Note the absence of lamins A and C in KE 37 cells and the presence of a 64-kd immunoreactive protein in both cell lines (arrowheads).

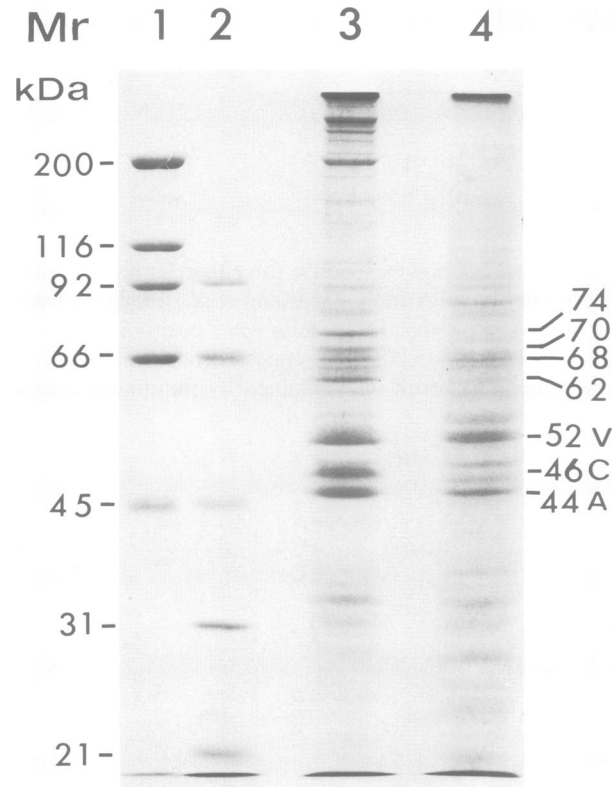

Fig. 3. SDS–polyacrylamide PAGE of nuclear lamina-IF fraction from HeLa (**lane 3**) and KE 37 cells (**lane 4**). **Lanes 1** and **2** contain mol. wt markers. Gels were stained with Coomassie blue. V, C and A referred to vimentin, cytokeratin and actin respectively.

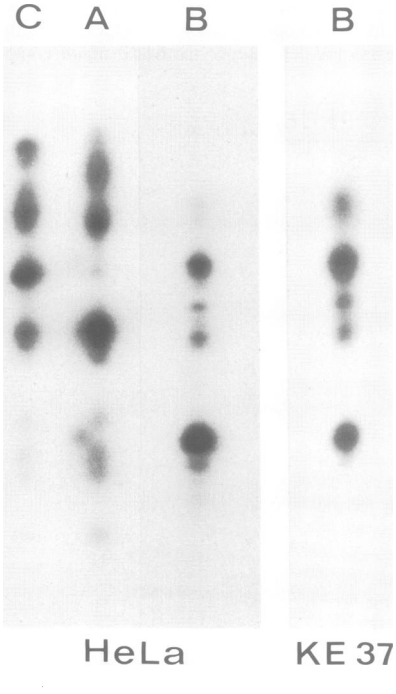

Fig. 4. Fluorographs of one-dimensional *S.aureus* V8 proteolytic peptides of lamins A, B and C from HeLa cells and lamin B from KE 37 cells. Lamins were obtained by immunoprecipitation with sera F and LS-1 of [^{35}S]-methionine-labelled nuclear proteins followed by electrophoresis on a 10% SDS–polyacrylamide gel (see Materials and methods). The peptide patterns display few differences between the two lamins B.

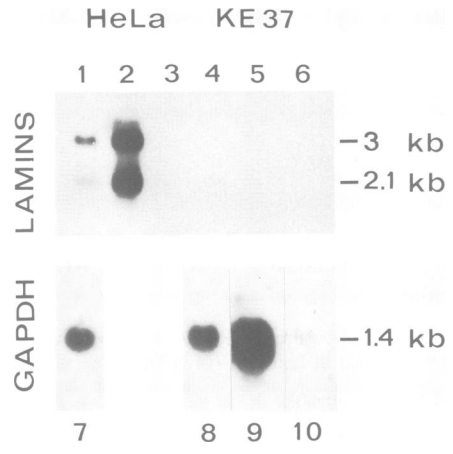

Fig. 5. Detection of lamins A and C RNA transcripts in HeLa and KE 37 cells. Total RNA from HeLa cells (**lane 1**) and KE 37 cells (**lane 4**), poly(A)$^+$ RNA from HeLa cells (**lane 2**) and KE 37 cells (**lane 5**) and poly(A)$^-$ RNA from HeLa cells (**lane 3**) and KE 37 cells (**lane 6**) were fractionated on a 1% agarose gel, transferred to nitrocellulose filters and hybridized with a human lamin A-specific probe. As a control of the RNA preparation, total RNA from HeLa cells (**lane 7**) and KE 37 cells (**lane 8**), poly(A)$^+$ RNA (**lane 9**) and poly(A)$^-$ RNA (**lane 10**) from KE 37 cells were hybridized with a rat GAPDH probe. Note the absence of lamins signals in KE 37.

of nuclear proteins extracted from these cells (Figure 1B). The only immunoreactive component revealed by anti-lamin A + C antibodies in KE 37 cells was a minor 64-kd polypeptide. This was also present in HeLa cells, although sometimes hidden by the strong signal from lamin C. Its isoelectric point, obtained by two-dimensional immunoblotting was close to 6, i.e. clearly more acidic than that of lamins A and C (Figure 2). The absence of lamins A and C in KE 37 cells is not due to selective proteolysis since identical results were obtained when cells were lysed directly by boiling in SDS sample buffer (Figure 1A). As this absence was documented with immunological techniques only, we compared the total protein content of lamina-IF fractions from HeLa and KE 37 by SDS gel electrophoresis. As shown in Figure 3, the overall spectrum of proteins was different in both cell types, particularly in the high molecular mass region ($M_r \geq 200$ kd) and in the 62- to 74-kd region. A 68-kd protein was present in both KE 37 and HeLa, while prominent proteins of 74, 72 and 60 kd were present in HeLa cells only. Thus proteins with molecular mass of lamins A and C seem to be absent from KE 37 nuclear lamina preparations.

To explore further the nature of the main lamin present in KE 37, we undertook a comparative structural analysis by mild proteolysis of the lamins of both cell lines. [^{35}S]Methionine-labelled proteins from KE 37 and HeLa cells were immunoprecipitated with sera F and LS-1, hydrolysed with *Staphylococcus aureus* V8 protease and the resulting peptides analysed by monodimensional SDS–PAGE. The results (Figure 4) revealed that the main lamin of KE 37 cells was indeed of the B family. We have not been able to isolate enough purified, radioactively labelled 64-kd polypeptide to perform a similar peptide mapping of this minor protein.

To investigate further the reasons for the absence of these proteins, we looked for the presence of their specific mRNAs. Total or polyadenylated RNA from HeLa and KE 37 cells was fractionated by electrophoresis in agarose gels containing formaldehyde and transferred to nitrocellulose paper. The

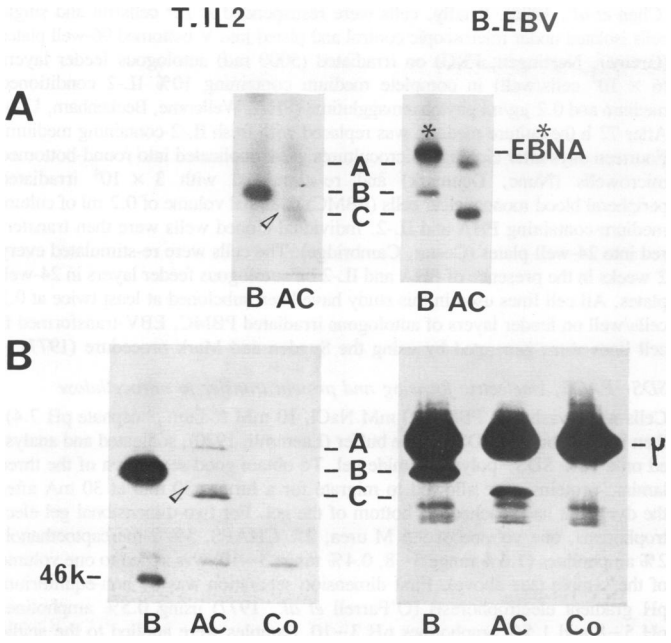

Fig. 6. Analysis of lamins in two lymphoid cell lines. Cloned T-IL-2 and B-EBV were studied by either immunoblotting (**A**) or immunoprecipitation (**B**). Proteins were probed with serum F (lamin B-specific) or serum LS-1 (lamins A + C-specific) or a mixture of normal control sera (Co). Note the weak signal obtained for lamins A and C in T-IL-2 and the presence of the 64-kd component (arrowhead). The 46-kd component detected by serum F in T-IL-2 (**panel B**) is a proteolytic fragment of lamin B which may arise when immunoprecipitation is performed in a low concentration (0.1%) of SDS. A proteolytic fragment of identical mol. wt has been shown to be generated from each mammalian lamin by treatment with chymotrypsin (Burke *et al.*, 1983). The 77-kd immunoreactive component obtained with the anti-lamin B serum on B-EBV cells (**panel A**, star) was also obtained by immunoblotting with the normal control sera (data not shown). It corresponds to the EBV nuclear antigen (EBNA). Antibodies to EBNA are present in >80% of normal human sera (Milman *et al.*, 1985) including the human anti-lamin B serum used here. The strong signal obtained for B-EBV with the three sera (**panel B**) is due to the binding of labelled Ig μ chain to protein A–Sepharose beads; this obscures the signal from lamin A but not from lamin B or C.

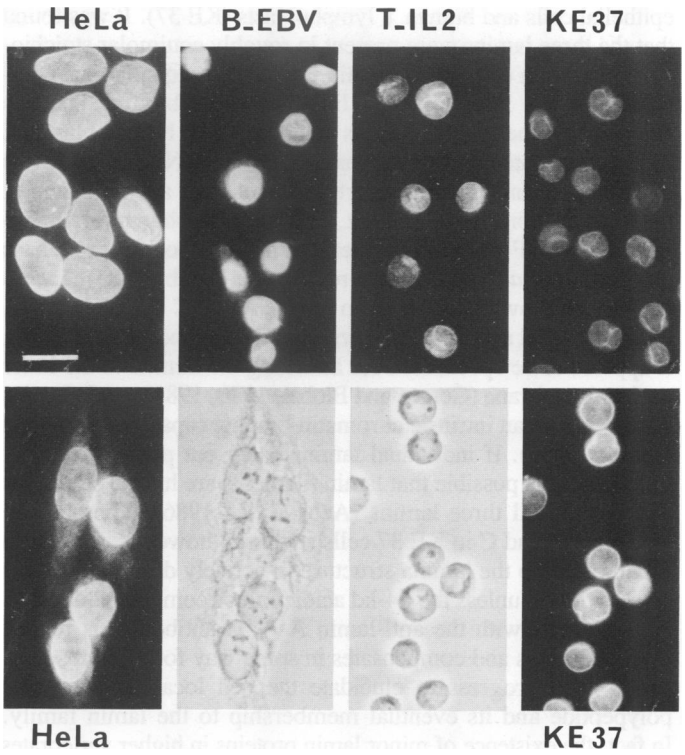

Fig. 7. Indirect immunofluorescence of HeLa cells and T lymphoid cells with anti-lamin A + C antibodies (**upper panel**) and anti-lamin B antibodies (**lower panel**). Note the weak fluorescence of T-IL-2 and KE 37 T lymphoblast nuclei with anti-lamin A + C antibodies which contrast with the strong fluorescence of HeLa and B-EBV. The strong fluorescence of KE 37 nuclei with anti-lamin B antibodies (lower panel) demonstrates that the fixation procedure allows a good penetration by antibodies. In the lower panel, HeLa and KE 37 are shown by phase contrast and immunofluorescence.

immobilized RNA was then hybridized with a nick-translated probe synthesized from a 1.3-kb *Bam*HI–*Hin*dIII fragment from the 3′ end of a cDNA coding for human lamin A (McKeon *et al.*, 1986; see Materials and methods). The results of such experiments are shown in Figure 5. Two bands with molecular lengths of 3 and 2.1 kb, which can be identified as mRNAs of lamin A precursor and lamin C respectively, were observed in HeLa cells. These sizes are similar to those obtained for the same molecular species in human cells (Fisher *et al.*, 1986; McKeon *et al.*, 1986). In T lymphoblast RNA none of these bands were detectable even in poly(A)$^+$ RNA. To take into account experimental variations in the amount of RNA transferred to the nitrocellulose, similar blots were hybridized with a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe, a key enzyme of glycolysis present in every tissue (Shonk and Boxer, 1964; Piechaczyk *et al.*, 1984). The 1.4-kb mRNA of GAPDH was found at similar levels in total RNA of HeLa cells and KE 37 cells and at high levels in the poly(A)$^+$ RNA prepared from KE 37. From these results it was concluded that the absence of lamins A and C in T lymphoblasts KE 37 was due to a lack of their specific mRNAs in these cells. This result also further ruled out the possibility that the minor 64-kd polypeptide detected by serum LS-1 is a proteolytic product of lamin A. On the other hand, this analysis with a human lamin A probe did not reveal any mRNA for this 64-kd polypeptide.

As both HeLa and KE 37 are transformed cells their difference in lamin expression is likely to be linked to cell type rather than growth state. Thus, the lamin content of two other types of human lymphoid cells was investigated: antigen-specific cloned T lymphocytes maintained in culture by interleukin 2 (T-IL-2) and B lymphocytes transformed by Epstein–Barr virus (B-EBV). Immunoblotting and immunoprecipitation experiments revealed signals of similar intensity for lamin B in both cell types but the intensity varied significantly for lamins A and C, being similar to that of lamin B in B-EBV and markedly lower in T-IL-2 (Figure 6). Finally, cells were tested by indirect immunofluorescence using the anti-lamin A + C antibody. Figure 7 shows that HeLa cells and B-EBV were strongly positive, in contrast with T-IL-2 and KE 37 T lymphoblasts. Although these cytological observations are basically in agreement with the biochemical data, we had expected a total absence of fluorescence in KE 37 cells. One possibility is that the weak immunofluorescence signal observed in this cell line could be due to the 64-kd minor protein detected by the anti-lamin A and C antibodies. However, we do not know yet if this protein is a component of the nuclear lamina or if it shares an antigenic determinant with lamins A and C.

Discussion

Using immunoblotting and immunoprecipitation, we have compared the amount of lamin A, B and C polypeptides in human

epithelial cells and human T lymphoblasts (KE 37). It was found that the three lamins were present in roughly equimolar stoichiometry in HeLa cells, while lamin B was the unique lamina component in the lymphoid cell line. Northern blotting analysis showed that the lack of lamins A and C in T lymphoblast KE 37 was correlated with the absence of their mRNAs. Thus levels of lamins A and C in this particular cell line are regulated at the transcriptional level and not, as it has been shown for vimentin, another IF protein, by the action of a specific proteinase (McTavish *et al.*, 1983; for a review see Traub, 1985). Lamin B has been shown to differ from lamins A and C by biochemical, immunological and functional criteria (Gerace and Blobel, 1981). It appears to be specialized for attaching the lamina to the inner nuclear membrane (Gerace and Blobel, 1980, 1981), while lamins A and C have an intrinsic chromatin-binding capacity (Burke and Gerace, 1986). If individual lamins carry out partially distinct functions, it is possible that lamin filaments are hybrid structures that contain all three lamins (Aebi *et al.*, 1986). The absence of lamins A and C in KE 37 cells suggests, however, that lamin B can build up the lamina structure in actively dividing somatic cells by itself, unless the 64-kd acidic minor component detected in these cells with the anti-lamin A + C antibodies is related to these lamins and compensates in some way for their absence. Work is in progress to elucidate the cell localization of this polypeptide and its eventual membership to the lamin family. In fact, the existence of minor lamin proteins in higher vertebrates has been recently demonstrated (Lehner *et al.*, 1986). This unusual lamina composition of the T lymphoblasts is reminiscent of that of the *Xenopus* oocyte. The nuclear envelope of *Xenopus* oocytes and early cleavage embryos is composed of a unique 66-kd lamin, which is unrelated to the other lamins (Krohne *et al.*, 1981; Benavente *et al.*, 1985; Stick and Hausen, 1985). However, in amphibians the presence of this particular lamin seems to be associated with the uncommon size, structure and physiology of the nuclei in oocytes and early embryos (Graham and Morgan, 1966; Krohne *et al.*, 1978).

Another conclusion that emerged from the peculiar lamin composition of KE 37 cells was that lamin expression in lymphoid cells may be subjected to important quantitative variations. This hypothesis was sustained by the additional analysis of the lamin composition of human cloned T lymphocytes and EBV-transformed human B lymphocytes. The lamin B level was nearly equivalent in both cell types, but the amount of lamins A and C varied to a large extent, from the level of lamin B in B-EBV to a low level in T-cells. It is interesting to note that the differences in the content of lamins A and C that we observe between the three lymphoid cells relates to their B or T origin. As all the lymphoid cells used here were actively dividing, this difference seems related to the cell type rather than to cell proliferation. This situation is reminiscent of that of vimentin in the same cells. Vimentin is expressed at different levels in B and T lymphocytes and this expression is abolished in tumor cells derived from B lymphocytes such as Burkitt's lymphoma (Dellagi *et al.*, 1983, 1984; Lilienbaum *et al.*, 1986). Using additional cell lines, we are currently investigating differentiation-dependent changes in lamin composition of lymphoid cells and their eventual functional significance.

Materials and methods

Cell culture

Human permanent cell lines used were either epithelial cells growing in monolayer (HeLa) or KE 37 T lymphoblasts growing in suspension (Mayer *et al.*, 1982).

The generation of human cloned cell lines has been described in detail elsewhere (Chen *et al.*, 1986). Briefly, cells were resuspended at 10^2 cells/ml and single cells isolated under microscopic control and plated into V-bottomed 96-well plates (Greiner, Nurtingen, FRG) on irradiated (5000 rad) autologous feeder layers (6×10^4 cells/well) in complete medium containing 10% IL-2 conditioned medium and 0.2 μg/ml phytohaemagglutinin (PHA, Wellcome, Beckenham, UK). After 72 h the culture medium was replaced with fresh IL-2-containing medium. Fourteen days after cloning, microcultures were duplicated into round-bottomed microwells (Nunc, Denmark) and re-stimulated with 3×10^4 irradiated peripheral blood mononuclear cells (PBMC) in a final volume of 0.2 ml of culture medium containing PHA and IL-2. Individual cloned wells were then transferred into 24-well plates (Costar, Cambridge). The cells were re-stimulated every 2 weeks in the presence of PHA and IL-2 on autologous feeder layers in 24-well plates. All cell lines used in this study have been subcloned at least twice at 0.3 cells/well on feeder layers of autologous irradiated PBMC. EBV-transformed B cell lines were generated by using the Sugden and Mark procedure (1977).

SDS–PAGE, isoelectric focusing and protein transfer to nitrocellulose

Cells were washed in PBS (150 mM NaCl, 10 mM sodium phosphate pH 7.4), then lysed in boiling SDS sample buffer (Laemmli, 1970), sonicated and analysed on a 10% SDS–polyacrylamide gel. To obtain good separation of the three lamins, proteins were allowed to migrate for a further 30 min at 30 mA after the dye front had reached the bottom of the gel. For two-dimensional gel electrophoresis, one volume of 9.5 M urea, 2% CHAPS, 5% 2-mercaptoethanol, 2% ampholines (1.6% range 5–8, 0.4% range 3–10) was added to one volume of the sample (see above). First dimension separation was by non-equilibrium pH gradient electrophoresis (O'Farrell *et al.*, 1977) using 0.5% ampholines pH 5–8 and 1.5% ampholines pH 3–10. Samples were applied to the acidic end of the gel and allowed to migrate toward the cathode for 6 h at 400 V. Second dimension electrophoresis was by 10% SDS–PAGE. Proteins were transferred to nitrocellulose by electrophoresis (Towbin *et al.*, 1979; Burnette, 1981), then probed with a 1:500 dilution of serum LS-1 (lamin A + C specific) or a 1:100 dilution of serum F (lamin B specific), followed by [^{125}I]protein A.

Immunoprecipitation

Cells were labelled *in vitro* for 6 h with 1 mCi/ml of [^{35}S]methionine in methionine-depleted RPMI-1640 medium containing 10% fetal calf serum. Nuclear ghosts were obtained from HeLa cells and KE 37 cells by different methods. HeLa cells were extracted for 2 min in PHEM buffer (45 mM Hepes–NaOH pH 6.9, 45 mM Pipes–NaOH pH 6.9, 10 mM EGTA, 5 mM $MgCl_2$, 1 mM PMSF, 10 mM *N*-ethylmaleimide) containing protease inhibitors (1 μg/ml leupeptin, 1 μg/ml pepstatin, 100 μg/ml aprotinin) and 1% Triton X-100, then washed in PHEM buffer. KE 37 cells were extracted for 10 min in PEM buffer (10 mM Pipes–NaOH pH 6.9, 1 mM EGTA, 5 mM $MgCl_2$, 0.1 M sucrose) containing 0.25% Nonidet P-40 (NP-40), *N*-ethylmaleimide and protease inhibitors as above. HeLa cell ghosts were lysed by a modification of the procedure of Gerace and Blobel (1980) in 100 mM Tris–HCl pH 8.3, 2 mM EDTA, 0.5% SDS, dispersed by sonication, then adjusted to 0.5% DOC, 0.5% NP-40, 5 mM iodoacetamide, 100 μg/ml aprotinin (DOC buffer). KE 37 cell ghosts were lysed in DOC buffer containing 0.1% SDS. Lysates were incubated overnight with a 1:50 dilution of anti-lamin sera. Each reaction was performed with 25×10^6 c.p.m. for HeLa and 50×10^6 c.p.m. for KE 37. Samples were mixed with protein A–Sepharose 4B beads and incubated with agitation for 2 h at 4°C. Beads were then washed four times with DOC buffer and finally boiled in SDS sample buffer. The eluted proteins were analysed on a 10% SDS–polyacrylamide gel and revealed by fluorography (Bonner and Laskey, 1974).

Cell fractionation procedure

Cell ghosts were prepared from HeLa and KE 37 cells as described above. A nuclear lamina-IF fraction was obtained according to Gerace *et al.* (1984). Briefly, cell ghosts were resuspended in 10 mM Tris–HCl, pH 7.5, 0.1 mM $MgCl_2$, 1 mM dithiothreitol, 0.5 mM PMSF, then digested with DNase I (20 μg/ml) and RNase A (20 μg/ml) for 1 h at 4°C, and collected by sedimentation through a 30% sucrose cushion in the same buffer. The pellets were washed with 1 M NaCl in the same buffer and collected by a similar centrifugation step. The insoluble fraction was resuspended in SDS sample buffer (Laemmli, 1970), sonicated, boiled for 3 min, precipitated in 90% (v/v) cold methanol and finally resuspended in SDS sample buffer.

Mild proteolysis

[^{35}S]Methionine-labelled nuclear proteins from HeLa and KE 37 cells were immunoprecipitated by sera F and LS-1, then separated on a 10% SDS–polyacrylamide gel. Regions of the gel corresponding to lamins A, B and C were excised from the gel and submitted to mild proteolytic digestion with *S.aureus* V8 protease (Miles Laboratory) according to Cleveland *et al.* (1977) using 2 μg/ml of enzyme and a 90-min incubation at 18°C. Separation of the proteolysed fragments was achieved on a 15% SDS–polyacrylamide gel. Visualization of the peptides was performed as described above.

RNA analyses

Total RNA from HeLa and KE 37 cells was extracted according to Auffray and Rougeon (1980). Poly(A)$^+$ RNA was isolated by selection on oligo(dT)−cellulose. RNA was fractionated by electrophoresis through a 1% agarose gel containing formaldehyde and transferred to nitrocellulose. Probes were either a 1.3-kb *Bam*HI−*Hin*dIII fragment from the 3′ end of a cDNA coding for human lamin A (cDNA-13 cloned in PUC9 by F.D.McKeon) or a 1.4-kb *Pst*I fragment of a cDNA coding for rat GAPDH (Fort *et al.*, 1985). They were labelled by nick-translation (Rigby *et al.*, 1977). Hybridization and high-stringency washing steps were performed according to Church and Gilbert (1984).

Immunofluorescence

HeLa cells, grown on cover-slips, were extracted with 0.5% Triton in PHEM buffer for 1 min. Lymphoid cells were centrifuged onto polylysine-coated cover-slips at 2500 *g* for 10 min, then extracted with 0.1% Triton in PHEM buffer for 1 min. After fixation in cold methanol, cells were incubated with anti-lamin A + C serum (1:500) or anti-lamin B serum (1:250) followed by fluorescein-conjugated goat anti-human Ig (Cappel, 1:100).

Acknowledgements

We thank Dr F.McKeon for the gift of anti-lamin A + C serum and lamins probes, Dr F.Danon for the gift of anti-lamin B serum, Dr J.-M.Blanchard for the gift of GAPDH probe, Drs F.Godeau, F.Gosti, B.Guiard, C.Klotz and B.Maro for their constant interest, Professor Damien Canape for moral support, Ms E. Houliston and Dr L.Sperling for critical reading of the manuscript, Ms J.Brizard for the typing of the manuscript, Professor D.Sandoz and M.Louette for photographic facilities. This research was supported by grant ARC N 6334. It was not supported by INSERM (CSS 3) nor by la Fondation pour la Recherche contre la Leucémie.

References

Aebi,U., Cohn,J., Buhle,L. and Gerace,L. (1986) *Nature*, **323**, 560−564.
Auffray,C. and Rougeon,F. (1980) *Eur. J. Biochem.*, **107**, 303−314.
Benavente,R., Krohne,G. and Franke,W.W. (1985) *Cell*, **41**, 177−190.
Bonner,W.M. and Laskey,R.A. (1974) *Eur. J. Biochem.*, **46**, 83−88.
Burnette,W.N. (1981) *Anal. Biochem.*, **112**, 195−203.
Burke,B. and Gerace,L. (1986) *Cell*, **44**, 639−652.
Burke,B., Tooze,J. and Warren,G. (1983) *EMBO J.*, **2**, 361−367.
Chen,L.K., Tourvieille,B., Burns,G.F., Bach,F.H., Mathieu-Mahul,D., Sasportes,M. and Bensussan,A. (1986) *Eur. J. Immunol.*, **16**, 767−770.
Church,G.M. and Gilbert,W. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 1991−1995.
Cleveland,D.W., Fischer,S.G., Kirschner,M.W. and Laemmli,U.K. (1977) *J. Biol. Chem.*, **252**, 1102−1106.
Dellagi,K., Vainchenker,W., Vinci,G., Paulin,D. and Brouet,J.-C. (1983) *EMBO J.*, **2**, 1509−1514.
Dellagi,K., Brouet,J.-C., Portier,M.-M. and Lenoir,G.M. (1984) *J. Natl. Cancer Inst.*, **73**, 95−99.
Fisher,D.Z., Chaudhary,N. and Blobel,G. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 6450−6454.
Fort,P., Marty,L., Piechaczyk,M., El Sabrouty,S., Dani,C., Jeanteur,P. and Blanchard,J-M. (1985) *Nucleic Acids Res.*, **13**, 1431−1442.
Gerace,L. and Blobel,G. (1980) *Cell*, **19**, 277−287.
Gerace,L. and Blobel,G. (1981) *Cold Spring Harbor Symp. Quant. Biol.*, **46**, 967−978.
Gerace,L., Comeau,C. and Benson,M. (1984) *J. Cell Sci. Suppl.*, **I**, 137−160.
Goldman,A.E,. Maul,G., Steinert,P.M., Yang,H.-Y. and Goldman,R.D. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 3839−3843.
Graham,C.F. and Morgan,R.W. (1966) *Dev. Biol.*, **14**, 439−460.
Guilly,M.N., Danon,F., Brouet,J.C., Bornens,M. and Courvalin,J.C. (1987) *Eur. J. Cell Biol.*, **43**, 266−272.
Krohne,G. and Benavente,R. (1986) *Exp. Cell. Res.*, **162**, 1−10.
Krohne,G., Franke,W.W. and Sheer,U. (1978) *Exp. Cell Res.*, **116**, 85−102.
Krohne,G., Dabauvalle,M.C. and Franke,W.W. (1981) *J. Mol. Biol.*, **151**, 121−141.
Laemmli,U.K. (1970) *Nature*, **227**, 680−685.
Lam,K. and Kasper,C. (1979) *J. Biol. Chem.*, **254**, 11713−11720.
Lazarides,E. (1982) *Annu. Rev. Biochem.*, **51**, 219−250.
Lehner,C.F., Kurer,V., Eppenberger,H.M. and Nigg,E.A. (1986) *J. Biol. Chem.*, **28**, 13293−13301.
Lilienbaum,A., Legagneux,V., Portier,M.-M., Dellagi,K. and Paulin,D. (1986) *EMBO J.*, **5**, 2809−2814.
Mayer,L., Shu Man Fu and Kunkel,H.G. (1982) *J. Exp. Med.*, **156**, 1860−1865.
McKeon,F.D., Tuffanelli,D.L., Fukuyama,K. and Kirschner,M.W. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 4374−4378.
McKeon,F.D., Kirschner,M.W. and Caput,D. (1986) *Nature*, **319**, 463−468.
McTavish,C.F., Nelson,W.J. and Traub,P. (1983) *Eur. J. Biochem.*, **130**, 211−221.
Miake-Lye,R. and Kirschner,M.W. (1985) *Cell*, **41**, 165−175.
Milman,G., Scott,A.L., Cho,M.S., Hartman,S.C., Ades,D.K., Hayward,G.S., Ki,P.F., August,J.T. and Hayward,S.D. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 6300−6304.
O'Farrell,P.Z., Goodman,H.M. and O'Farrell, P.H. (1977) *Cell*, **12**, 1133−1142.
Ottaviano,Y. and Gerace,L. (1985) *J. Biol. Chem.*, **260**, 624−632.
Piechaczyk,M., Blanchard,J.M., Marty,L., Dani,C., Panabieres,F., Sabouty,S.E., Fort,P. and Jeanteur,P. (1984) *Nucleic Acids Res.*, **12**, 6951−6962.
Rigby,P.W.J., Dieckmann,M., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237−251.
Shonk,C.E. and Boxer,G.E. (1964) *Cancer Res.*, **24**, 709−721.
Stick,R. and Hausen,P. (1985) *Cell*, **41**, 191−200.
Sugden,W. and Mark,W. (1977) *J. Virol.*, **23**, 503−508.
Towbin,H., Staehelin,T. and Gordon,J. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4350−4354.
Traub,P., ed. (1985) *Intermediate Filaments: A Review*. Springer-Verlag, Berlin.

Received on April 23, 1987; revised on September 8, 1987